Docket No.: 273243US0PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF: :

Syunichirou OSHIMA, et al. : EXAMINER : TONGUE, L. J.

SERIAL NO: 10/538,882 :

FILED: JUNE 14, 2005 : ART UNIT: 1645

FOR: VACCINE FOR COLD-WATER DISEASE IN FISH

DECLARATION UNDER 37 C.F.R. § 1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

Sir:

Now comes Syun-Ichirou Oshima who deposes and states that:

1. I am a graduate of Kochi University, and received my Ph. D. degree in the field of Medicine, in the year 1999.

2. I have been employed by Kochi University, for 7 years in the field of Microbiology, Immunology.

3. I understand the English language or, at least, that the contents of the Declaration were made clear to me prior to executing the same.

4. The following experiments were carried out by me or under my direct supervision and control.

5. The following experimental procedure was followed:

1) A platinum loop of Flavobacterium psychrophilium G3724 from a frozen culture (~10^9 CFU/ml) was inoculated on 50 mL modified cytophaga culture medium in Erlenmeyer flask (300 mL).

2) The bacteria were cultured with shaking (100 rpm) at 15°C for 48 hours.

3) 2.5 mL of the culture medium was inoculated on 1 L modified cytophaga culture medium in Sakaguchi flask (2 L).

4) The culture medium was cultured with shaking (100 rpm) at 15 or 20 °C, while the number of cells and OD 600 were measured sequentially.

6. None of LeFrentz et al, Masunari et al and Rahman et al, clearly describe the culture condition including the starting cell number. Thus, we started culturing with commonly used condition, namely used cells from frozen stock of about 10^9 CFU/ml.

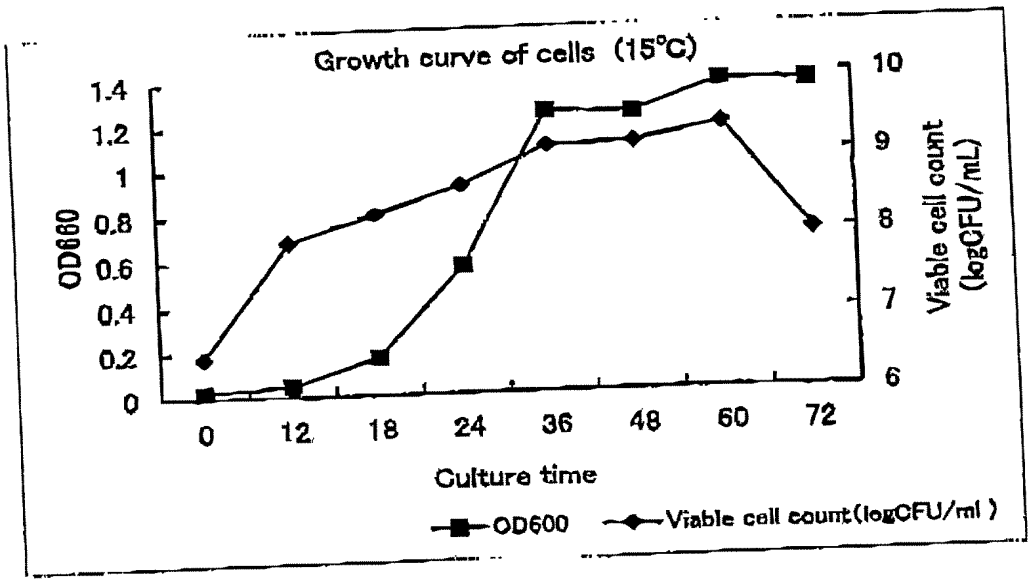
In LeFrentz et al, the bacteria were cultured in TYES medium at 15°C for 72 hours. In Masunari et al, the bacteria were cultured in modified cytophaga medium at 18°C for 3 to 3.5 days (i.e., 72-90 hours). And, in Rahman et al, the bacteria were cultured in MCY (modified cytaphaga) medium at 20°C for 48 hrs and in CGY medium at 20°G for 24 hrs (totally for 72 hours).

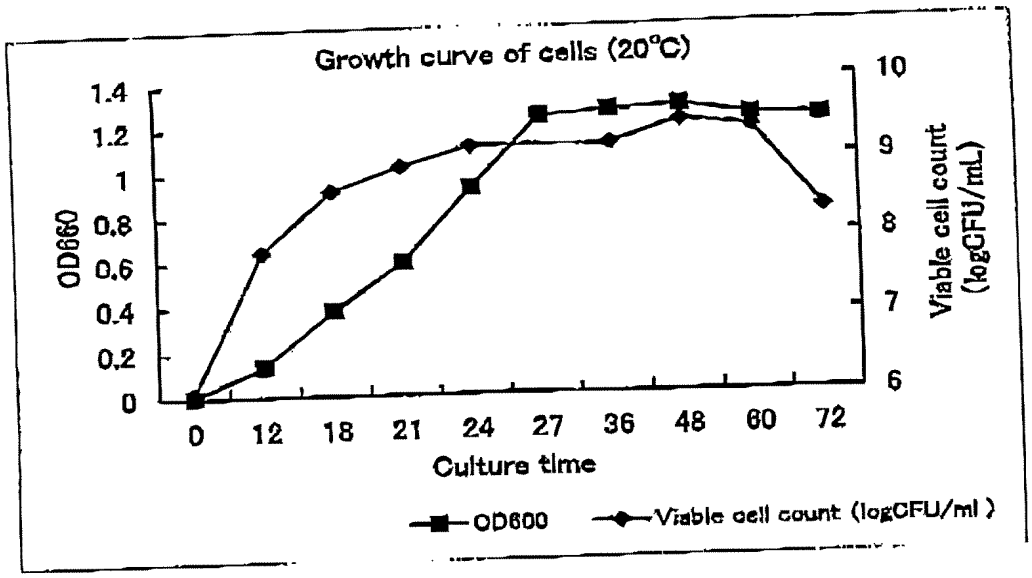
The culture media used in Masunari et al and Rahman et al (modified cytophaga) were the same as that used in the present invention (MCYT). TYES used in LeFrentz et al and CGY used in Rahman et al were also similar to MCYT used in the present invention as shown in Table 1.

Component	TYES	Modified cytophaga	CGY
Tripton	0.4%	0.2%	
Caditone			0.5%
Yeast Extract	0.04%	0.05%	0.1%
Calcium chloride	0.05%	0.02%	
Magnesium sulfide	0.05%		
Sodium acetate		0.02%	
Meat extract		0.02%	
Glycerol			0.5%

Proliferating potential of bacteria in each culture can be expected to be at the similar level since the culture conditions are quite similar to each other.

7. The results of these experiments are illustrated by the following figures:





8. In this experiment, we studied proliferating potential of the bacteria in modified cytophaga (Masunari et al's), and found that the culture reached stationary phase within about 24 to 36 hrs. As stated in paragraph (6), above, in LeFrentz et al, the bacteria were cultured

in TYES medium at 15°C for **72 hours**; in Masunari et al, the bacteria were cultured in modified cytophaga medium at 18°C for 3 to 3.5 days (i.e., **72-90 hours**); and, in Rahman et al, the bacteria were cultured in MCY (modified cytaphaga) medium at 20°C for 48 hrs and in CGY medium at 20°G for 24 hrs (totally for **72 hours**). Thus, the result of the experiments herein and illustrated in the figures appearing in paragraph (7) establish that bacteria used in LeFrentz et al, Masunari et al, and Rahman et al were in stationary phase, rather than logarithmic phase as those used in the present invention.

Moreover, as the culture conditions of the cited references were not clearly disclosed, the skilled artisan would not expect the advantageous effect of the bacteria in logarithmic phase as used in the present invention from the description of the references.

9. I declare further that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

10. Further Declarant saith not

S. Oshima

Name:

September 14, 2007

Date